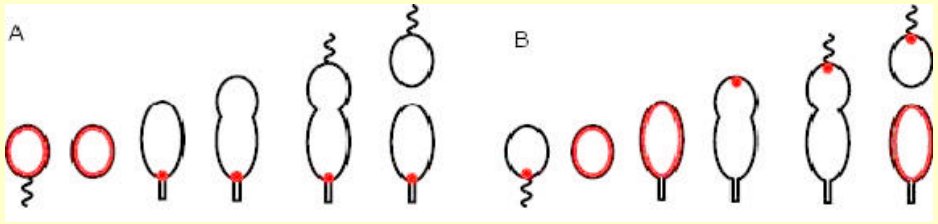
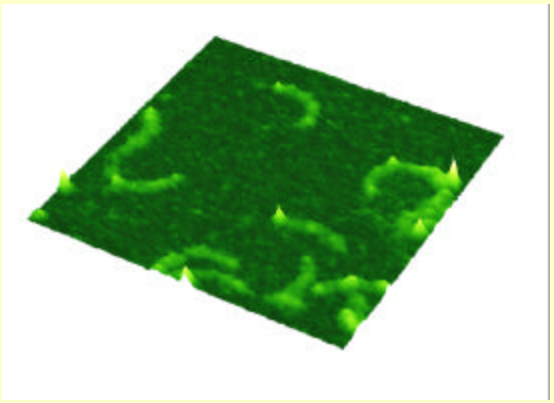
Advanced Microscopy for Protein Localization in Caulobacter





W. E. Moerner, Matt Paige, S. Nishimura
Ellen Judd, Lucy Shapiro
Stanford University
supported in part by DARPA

The Problem

•Differentiation and asymmetric cell division in Caulobacter have been shown to be accompanied by a pattern of protein localization

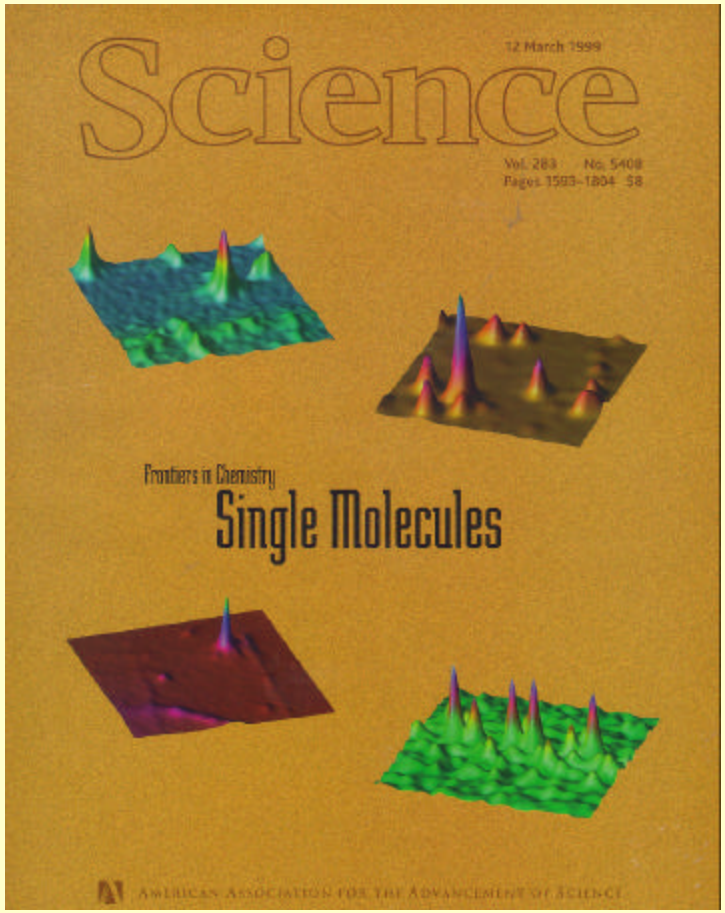
•Detecting location of proteins in live bacteria can be done by fluorescent labeling with GFP

•Research Challenge:

Determine real-time location of proteins such as divJ, PleC, in a 1μmx2μm sized object. Requires subwavelength resolution and extreme sensitivity

•Plan: Utilize ultimate sensitivity of single-molecule microscopy coupled with selected near-field and ultraresolution microscopies

The Promise



This cover of Science (March 1999) shows imaging of individual molecules by the Moerner Lab by a variety of techniques, both at low temperatures, as well as in room temperature biomolecular environments.

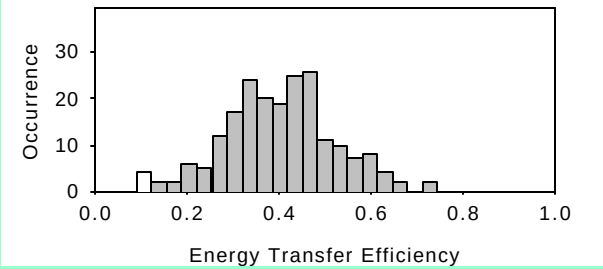
Key idea: Observing individuals removes ensemble averaging, allowing exploration of

- static heterogeneity (different folds, structures, local environments, ...)
- dynamic heterogeneity (different nucleotide states, activities, ...)

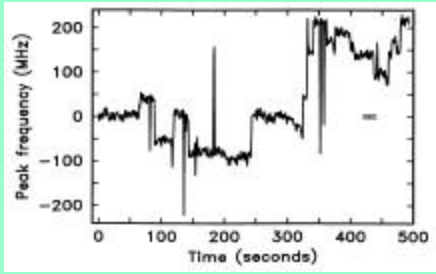
Optical Study of Single Molecules (1.66 ym)

We *optically* study the spectroscopy, orientation, motion, and dynamical behavior of *single, individual* chromophores in *complex* environments: solids, liquids, proteins, ... Why is this possibly useful?

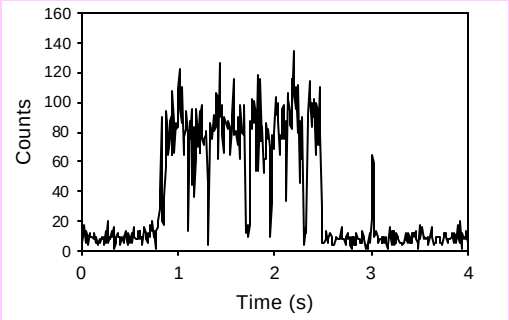
No ensemble averaging: direct observation of (static and dynamic) heterogeneity by measuring the full distribution



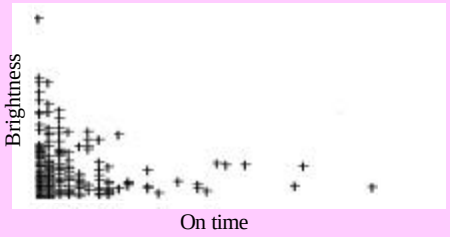
Extreme sensitivity to immediate local nanoenvironment; sense local strain field changes (spectral diffusion) or local optical field



Time-dependent state changes directly observable, without synchronization, and can detect rare intermediates

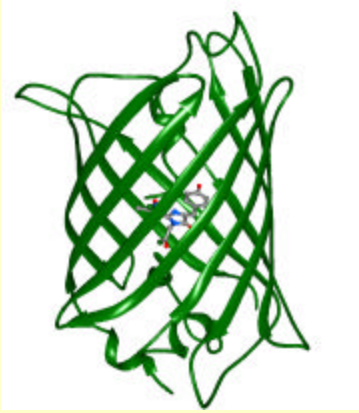


Statistical correlations possible: can plot lifetime vs linewidth, brightness vs on-time, ...



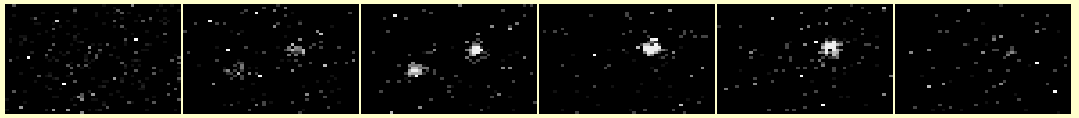
Quantum optics: photon antibunching, nonclassical light, ... for single molecule trapped by the solid

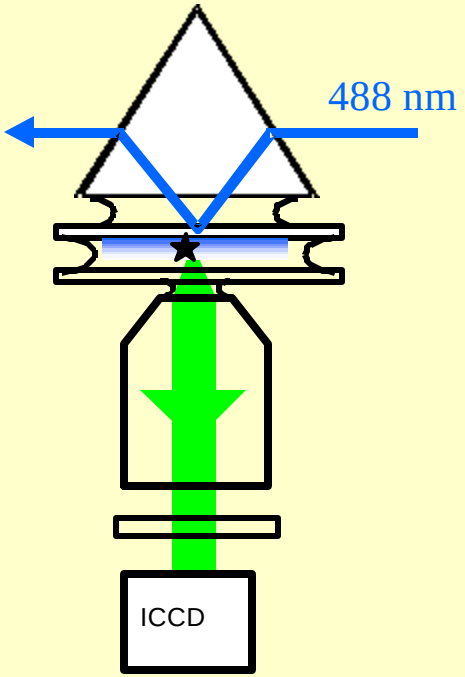
Moerner Group



Blinking and Switching Dynamics for Single Copies of Green Fluorescent Protein

3x2 μm, 100 ms frames





Wide-field TIR microscopy, water-filled agarose gels

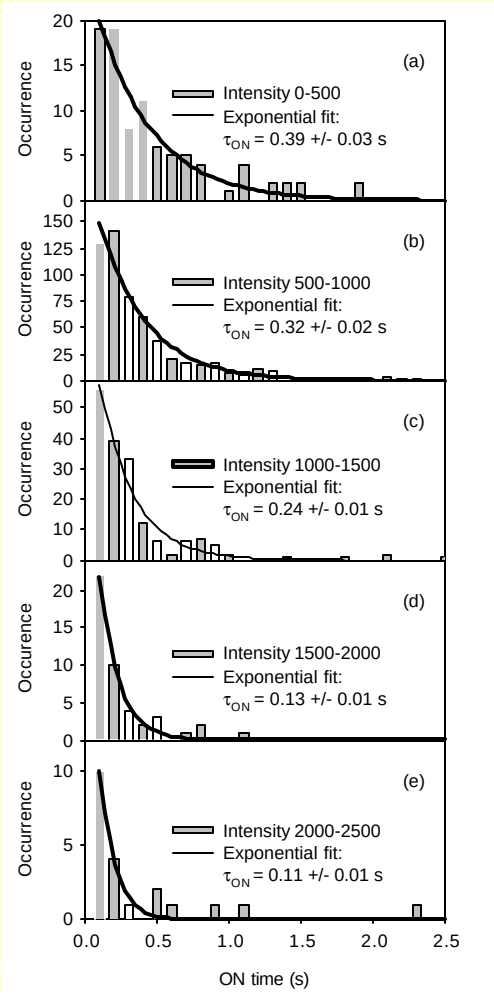
Emission characteristics: Short on-time, long off time, reversible

On-time distribution scales inversely with intensity

No pH dependence

Consistent with reversible bleaching with q.e. 10^{-5}

Probable mechanism: photoisomerizaton

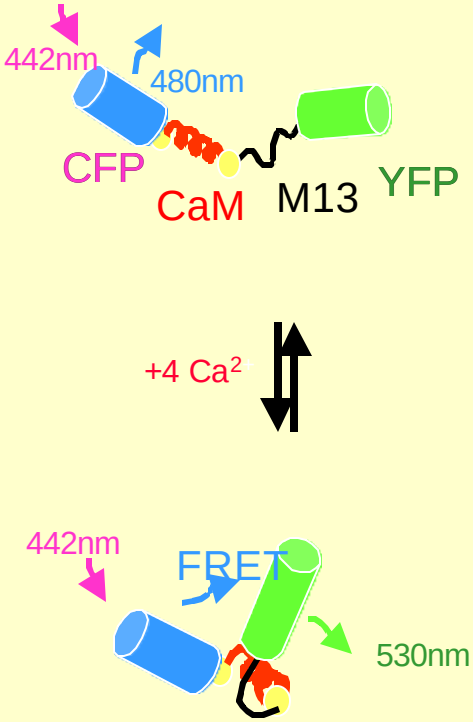


Nature (1997) 388, 355
J. Phys. Chem. (1999) A103, 1553

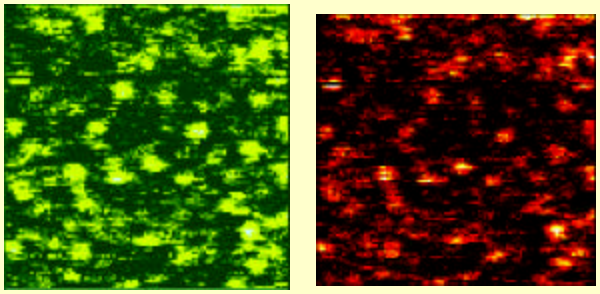
Detection of Local Calcium Concentrations by Fluorescence Resonance Energy Transfer From Single Molecules

Sophie Brasselet, Erwin Peterman, Atsushi Miyawaki*, Roger Tsien*, and W.E. Moerner

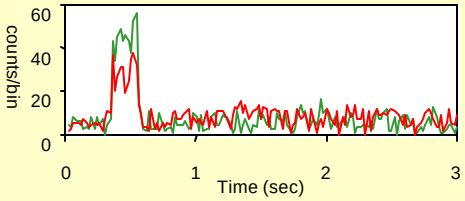
Dual-GFP "Cameleon"



2-color confocal microscopy

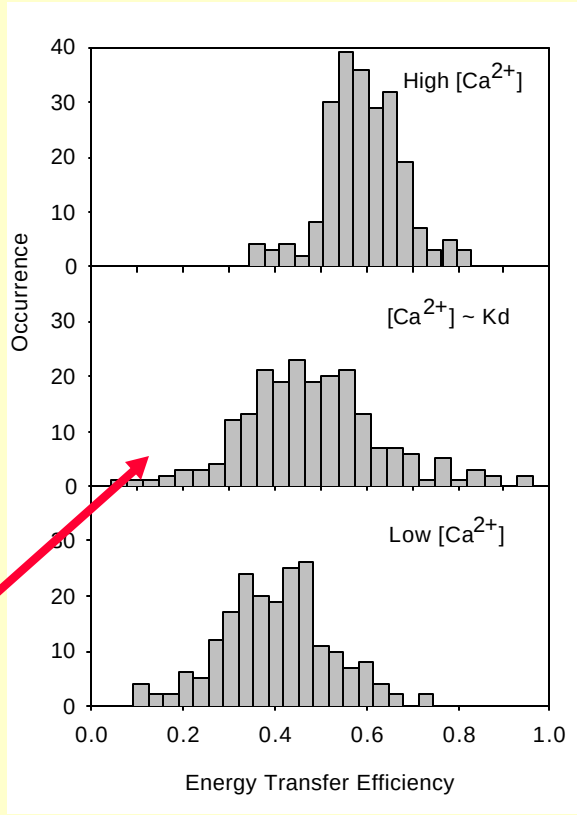


SM time traces: D and A



excess width at intermediate [Ca++] due to binding kinetics

single-molecule histograms



*Collaborators from UC San Diego

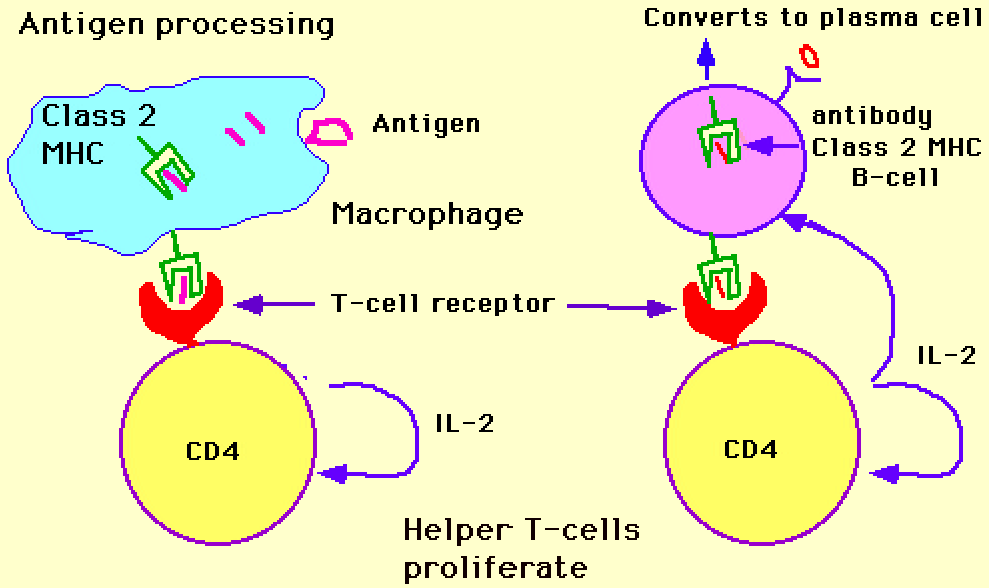
See J. Phys. Chem B 104, 3676-3682 (2000).

Imaging Single Molecules *in vivo*

Recent advance (unpublished):
Imaging of single MHCII proteins on live cells

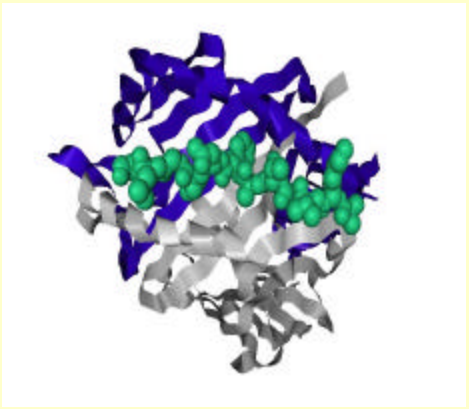
S. Brasselet, S. Nishimura, WEM
M. Vrljic, H. McConnell

Background:



from The Biology Project, developed at
The University of Arizona.

extracellular view



Fremont, et al. Science 1996, 272: 1001

Key idea: peptide off-times are ~200 hr; label peptides with fluorescent tag via -SH